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Someichirô MASUDA*: **Studies on the *Caulobacter* in Japan****

増 田 染 一 郎*: 日本産カウロバクターの研究**

On the present organism, Several reports have been published by Jones (1905), Omeliansk (1914), Henrici and Johnson (1935).

Following these studies, it is known that Houwink and Iterson (1950) observed *Caulobacter* in the electron microscopic preparation of the contamination, and Houwink (1951, 1955) succeeded in pure culture and published the many morphological electron microphotographs.

According to Houwink's great helpness for the studies, Bowers & al. (1954), Kandler & al. (1954), Houwink (1952), Hund and Kandler (1956) reported separately their observations.

The interesting point of Houwink's observation is the fact that *Caulobacter* is parasitic on other bacteria.

Bowers & al. (1954) observed the perfectly same organism with Houwink's one on the morphology by the electron microphotograph, but could not observe the parasite character. From the first experiment of the physiological nutrition which was done by Grula & al. (1954), the nutrition was said to have relations with glucose and riboflavin, but such a result of study is still incomplete. Then, Hund and Kandler (1956) studied on the nutritional requirements by the method of gas metabolism. The present study deals with the morphology, cultural characteristics, physiology, and parasitic character.

Isolation Samples were collected, using 50 ml sterile glass tube, from the several puddles in the stalactite grottoes at Izuru in Tochigi Pref. in Sept., 1954.

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After the samples had been brought back to the laboratory, two sterile cover glasses were thrown into them, and glass tubes were set in quiet at room temperature. After 7 days, the one glass was picked up, dried, and stained by Loeffler's alkaline methylene blue. After recognizing stalked bacteria from the observation by the light microscope, the one more cover glass was picked up in quiet, and was set into the sterilized water of 5 ml to make the washed suspension of stalked bacteria which attached on the cover glass. Then, 0.2–0.4 ml of the suspension was dropped on every three plates of 0.5 % pepton agar, and spread well, and incubated at 25°C for 7 days.

After the observation by the light microscope about all grown colonies, using 1 % pepton agar plate, spreaded with the above method, and at 25°C after 5 days isolated pure culture of stalked bacteria.

Cultural characteristics and morphology The organism grows well on 1 % pepton agar plate. This medium was used soon after the sterilization. After 4 days, the surface colonies are round, smooth, slightly raised, glistening, edges entire at 150 X, non-visicid, almost translucent, light grayish-white, ranging from 0.1–0.5 mm and averaging 0.2–0.3 mm in diameter. The cells are Gram-negative. The cells was observed on slide cultures, using a phase microscope. The cells are slightly curved, vibrio-like, ranging from 0.4–0.6 μ in diameter and 1.3–3.2 μ in length. The cells occur singly, in pairs or short chains, frequently filaments. Non-sporeforming. Not ensheathed. Stalks are slender, flagellum-like, often attach to the substrate by a button-like holdfast, unbranched. Width of stalks is approximately 0.2μ and the length averaging 2.0–3.5 μ. In some individuals stalks are very short or absent (Fig. A). The cells multiply by transverse binary fission. The parent cells are stalked, and flagella are formed both at the free end of stalk of cell and at the other end of the same cell which has had no stalk. This appears to be similar to or identical with the course that observed by Houwink and Iterson (1950), Houwink (1951) and Bowers & al. (1954). After the multiplication of cell is completed, daughter cell seems to break away from the parent cell and to be actively motile (Fig. C). Then a stalk of daughter cell starts to form at the same end of the cell as the flagellum, and the flagellum is formed at the free end of the stalk. In some stalked cell, the filament-like things radiate from the base of the stalk (Fig. D). This filament-like things were observed by Houwink (1951, 1955), Bowers & al. (1954) too, but no one has ever been to answer exactly whether they are flagella or only a filaments, and what task they do.

Houwink (1955) reported that in a liquid pepton medium the cultivation was not always successful, but in the liquid pepton medium added riboflavin the cultivation was always successful. In a liquid pepton medium added 0.01 % riboflavin, at 25°C, after 5 days, pellicle was formed. The pellicle was very brittle, and sunk easily even by a little vibration and sediment was formed. Observing the pellicle by light microscope, it could be always seen that a pole of stalk touched each other as Fig. F, and each cell became together in the chestnut-bur-shape (Fig. E).

Jones (1905), Henrici and Johnson (1935), Houwink and Iterson (1950), called the above state as the rosettes, but it seems to be the bur-shape group. The developmental cycle of the stalked bacteria was studied in hanging-drop-cultures of liquid pepton added 0.01 % riboflavin, using a phase microscope. After 3 days, cells were 0.5–0.8 μ in width, 1.5–3.8 μ in length; and stalks were approximately 0.3 μ in width, averaging 2.0–4.5 μ in length. After 4 days, every cell started to from the chestnut-bur-shape. It was frequently found that the cell which was constricted at the central portion and seemed to be multiplying off, was the member of the chestnutbur-group. After 6 days, the cells became 3.0–6.5 μ in length but not altered in width. The stalk were 4.1–13.0 μ in length, and the end of the stalk attached each other (Fig. F), but as the stalk curved irregularly, cell did not sit radiately from the center but toward the irregular way (Fig. F). From the free end of cell that had stalked at the one end, new stalk started to grow (Fig. G). After 8 days, the part constricted at centre was streched and altered to the new stalk which connected two cells (Fig. H). After 14 days, some cells became 6.0–21.0 μ in length. Some of them were going to multiply, constricting at several parts (Fig. I). The stalk became enervated, curved irregularly, and winded round itself or the other. After 18 days, some cells were more than 35 μ in filamentous shape. As the filamentous cell became long, the stalk seemed to become short (Fig. J). After 25 days, it became more than 31–85 μ in length, and showed the state which it could not be thought the stalked bacteria. After 45 days, the alternation was not observed. From the observation of the filamentous cell stained by Loeffler's alkaline methylene blue, it was stained like the chain of the rods of various sizes. This filamentous cell was observed, inoculating on 1 % pepton agar plate. The results of the inoculation were the next. After 8–12 hours, some constricted part occured (Fig. K). After 12–24 hours, multiplied to each cell which was 0.4–0.9 μ in diameter and 1.3–2.8 μ in diameter and 1.3–2.8 μ in length (Fig. K). Stalked cells were about half of all cells in sight, and stalks were short that was 0.7 μ in length 0.3 μ in width. Some of stalked cell was constricted at the part which adjoined on the sphere-shape swelling (Fig. L). Cells with the above swelling had stalk always on the end of cells. Approximately, some cells were observed to multiply strangely like this (Fig. L). This strange cell, after 36 hours, multiplied, and the central swelling altered to the short stalk of new cell.

The stalk was determined not to be secretion but an outgrowth of cell, from the obsrvation of that the cells were chained by the stalks and that at the multiplication the central swelling altered to the stalk. And it appears to have the same cover membrane as the cell wall of *Caulobacter*. The result of the present author's experiment is supported by Bower's saying that the filament like cytoplasm of the cell extends evidently into the stalk. The same central filament, like Fig. M, as Bower's one shown on the unshadowed preparation, was proved by the state which the outgrowth of the stalk was broken and the filament exposed nakedly like Fig. N, and it was proved too the outgrowth of the stalk was different from the central filament.

The hold-fast appears to be a viscous secretion from the tube-shape stalk (Fig. B. Arrow). When the stalked bacteria formed the chestnut-bur-shape group, they attached each other by the viscous material (Fig. F, Arrow).

Colonies grown on nutrient broth agar are round, slightly raised, smooth, glistening, finely granular in the center, grayish-white, up to 0.8 mm in diameter.

On nutrient broth agar slant the growth is abundant, filiform, raised, brownish yellow, glistening and non-viscid.

Physiology Indol is not formed on 2 % pepton agar of pH 7.0 after 4, 7, 9 or 15 days, as determined by the technic of Ehrlich-Böhme. 0.1 % KNO_3 in 1 % pepton agar (pH 7.0) is reduced after 6 days, as shown by the sulphanic acid -α-naphthylamine test. Litmus milk; No visible change. H_2S is not formed, inoculated on 2 % pepton agar slant (pH 7.0) and tested by lead acetate test strips method. The ability of sugar fermentation was determined by means of Durham tube with a medium consisting of 1 % pepton, 0.5 % of the sugar, pH 7.0 with B. T.B indicator to demonstrate acid production. As is indicator below, acid is produced from glucose, sucrose, maltose, but not produced from arabinose, rhamnose, xylose, fructose, galactose, mannose, lactose, raffinose, starch, inulin, dextrin, glycogen, glycerol, adonitol, sorbitol and mannitol.

The optimum pH and temperature ranges for growth were determined on nutrient broth agar slant of pH values of 5, 6, 7, 8 and 9. Culture were incubated at 20°C, 27°C, 32°C and 37°C. The growth was observed at 24 hours intervals with the naked eyes. As the result was shown by following table 1, the best condition was at 27°C and pH 7–8. So, the growth temperature of this study was always incubated at 27° C. and all media were adjusted to pH 7.5.

Salinity range for growth was determined, after the observation, by the naked eyes, the growth on the pepton agar added 1 %, 2 %, 3 %, 4 % NaCl and added nothing. This experiment brings on the following; the growth was done up to 1 % NaCl from 0 % but the growth condition was on the pepton agar without adding NaCl was slightly toxic.

Taxonomy Jones (1905) reported an organism isolated from the city water supply and from the sewage in Chicago, without proposing the name.

Nine years later, Omeliansky (1914) proposed the name *Bacillus flagellatus* to an organism that was little different from Jones' organism on the cultural characteristics. But his description is incomplete since he did not study by pure culture.

After then, Henrici and Johnson (1935) rejected the name given by Omeliansky on the basis that it could not be established, and the single polar flagellum described by him was actually stalk, although their organisms by the observation using the light microscope without the pure culture seem to be the same with the organism of the former two men. And Henrici and Johnson established the genus *Caulobacter* with a monotypic *Caulobacter vibrioides*.

The present author's organism agrees with the description by Henrici and Johnson and so it was identified with *Caulobacter vibrioides*.

Parasitism If *Bacillus megaterium* is cultivated in a diluted liquid pepton medium simultaneously with young culture of *Caulobacter*, *Caulobacter* may be observed to attach *Bacillus megaterium* by the end of the stalk. After a while this *Bacillus megaterium* occured cytolysis (Fig. O). It seems that it is the very true culture, and it may be observed repeatedly. As *Caulobacter* does not grow at 37°C, on the third and the seventh day of mixed culture with *Bacillus subtilis*, the number of cells was counted, using the nutrient broth agar plate culture by means of the dilution method. On the seventh day *Bacillus subtilis* decreased to 1/3 in number of the third day. The young culture of *Caulobacter* is parasitic very well than the old culture. It was always parasitic on *B. mycoides*, *B. mesentericus*, *B. cereus* and occured lysis.

Houwink (1951, 1955) recognized that *Caulobacter* was parasitic on bacteria, by the two observation which was done on the preparation of bacteria already lysed partly, using the electron microscope.

As to same species of bacteria, this parasitic characteristic has brought the same result as Houwink's, but no one has ever tried to let it be parasitic on the other micro-organism.

Growth condition on nutrient broth agar slant.

Incubation temp.	pH	Incubation time in days.				
		1	2	3	4	5
20°c	5	−	−	−	−	−
	6	−	−	+	+	+
	7	−	+	+	++	++
	8	−	+	+	++	++
	9	−	−	−	+	+
27°c	5	−	−	−	−	−
	6	−	+	+	+	++
	7	−	+	++	++	+++
	8	+	+	++	+++	++++
	9	−	−	+	+	+
32°c	5	−	−	−	−	−
	6	−	+	+	+	++
	7	−	+	++	++	+++
	8	+	+	+	++	++
	9	−	−	−	−	+
37°c	5	−	−	−	−	−
	6	−	−	−	−	−
	7	−	−	−	−	+
	8	−	−	−	−	−
	9	−	−	−	−	−

Table 1. These degree were expressed in symbols as follows;— (no growth), + (scanty growth), ++ (moderate growth), +++ (abundant growth), ++++ (very abundant growth).

On observating the mixed culture with *Candida albicans* by hanging drop culture, it was seen that the ends of the stalks of many *Caulobacer* attached to *Candida albicans*. After more 30 days, *Candida albicans* occured plasmolyse and lysis, *Caulobacter* at that time grew in a filamentous form.

Distribution Well water was found to be the good habitat of *Caulobacter vibrioides*. As the well water is influenced physically or chemically by the various surrounding conditions, the following type was selected as an object for this study; that was covered, vertical, concreted the inside and set the well pump. Sample water was collected in the sterile glass tube by well pump. After the observation by the same method as the one of the isolation, it was evidently shown that *Caulobacter vibrioides* lives in 69 wells out of 76 sample well-waters.

Summary

1) The organism which was isolated from the water in the stalactitic grottoes proved to be similar to an organism that was first described by Jones in 1905, and was identified with *Caulobacter vibrioides*. The isolation of this organism is the first in Japan.

2) The stalk of *Caulobacter vibrioides* is not a secretion of the cell, but a part of the cell and an appearance of cell wall which altered in a tube-like shape. These stalks are formed, sometimes, at both ends of the cells, and cells are frequently chained to each other by the stalks.

3) *Caulobacter vibrioides* is parasitic on *B. subtilis*, *B. megaterium*, *B. cereus*, *B. mycoides*, *B. mesentericus* and *Candida albicans*, and lysed.

4) In 69 samples out of 76 well water, *Caulobacter vibrioides* lived.

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□今関六也・本郷次雄: **原色日本菌類図鑑** 出版所 保育社 定価 1,200 円

保育社の原色図鑑シリースの第 23 として 11 月 10 日に本書が出版された。両著者は数少い日本の高等菌類学者であり，今関氏はどちらかと申せば硬質菌に得意であり，本郷氏は軟質菌専門であり，ここに硬軟相和して両者ともお得意の水彩画に多くのカラースライドを添へ，386 種を図説された。中には専門画家の筆になるものも少くない。今迄日本で図示された普通のキノコの他に，名のみ知られて図のなかつたキノコが全体の 20% 位を占めて紹介されているのも有難い。印刷も先づ申分ない。ことにバツクの色，菌体の縁取りなど良好である。学名は Singer 其他による新しい方式が採用され，巻末には多くの写真とともにキノコの概説がある。(小林)

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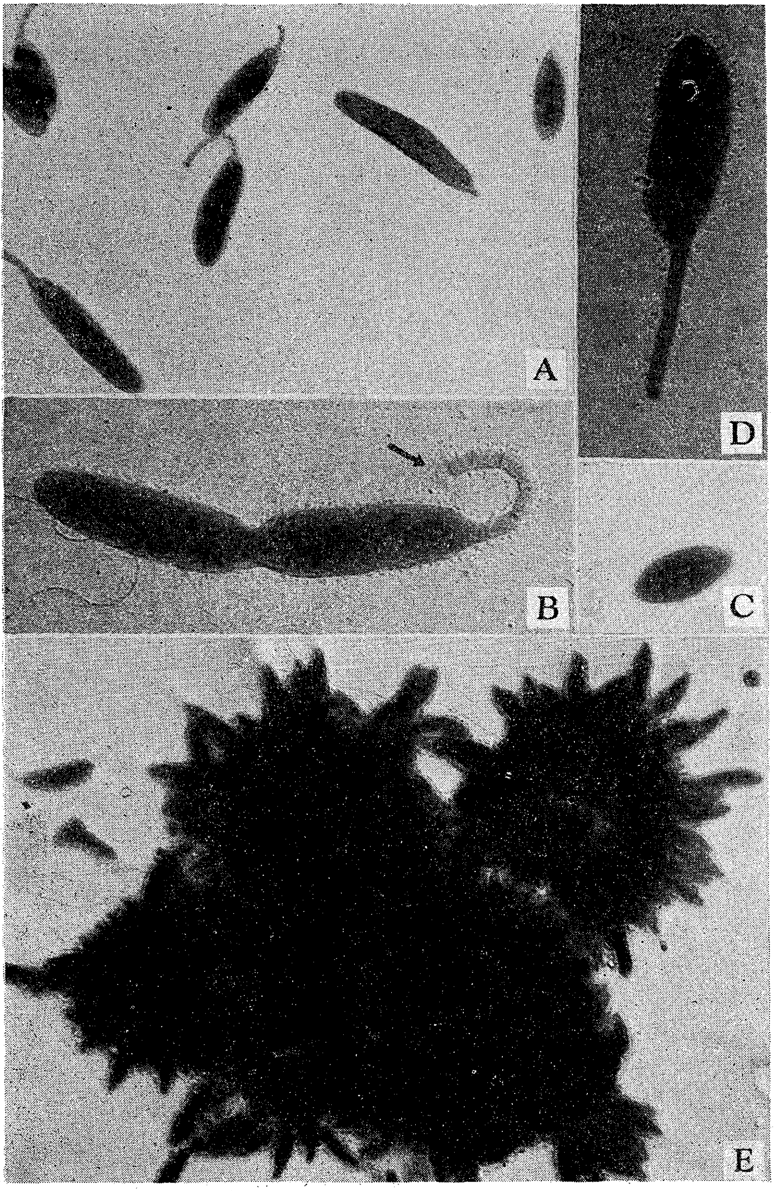
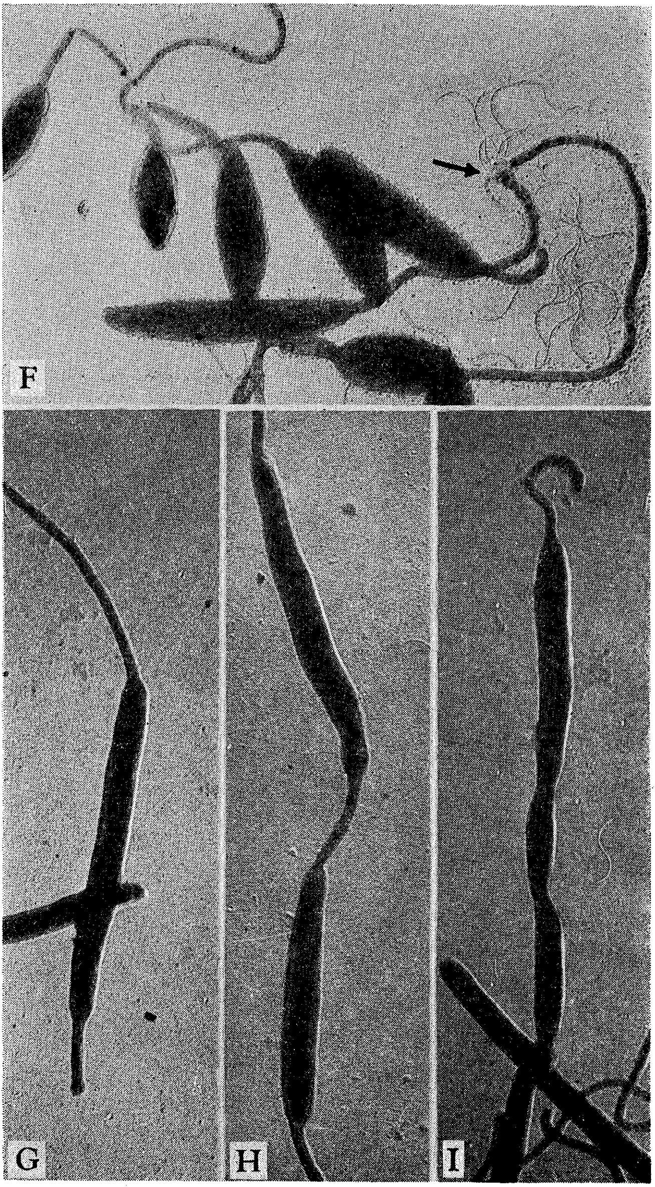


Pl. I. A. Electronmicrograph, chromshadowing, *ca* ×8,500. Young culture on 1% pepton agar plate: the different size of cells presented.
B. Electronmicrograph, chromshadowing, *ca* ×17,000. Later stage in transverse fission.
C, Electronmicrograph, chromshadowing, *ca* ×12,000. New cell has not yet grown a stalk, but still possesses a flagellum.
D. Electronmicragraph, chromshadowing, *ca* ×17,000. Filament-like things radiate from the base of the stalk.
E. Electronmicrograph, chromshadowing, *ca* ×3,400. Chestnut-bur shape groups in liquid pepton medium added 0.01 % riboflavin, after 5 days.

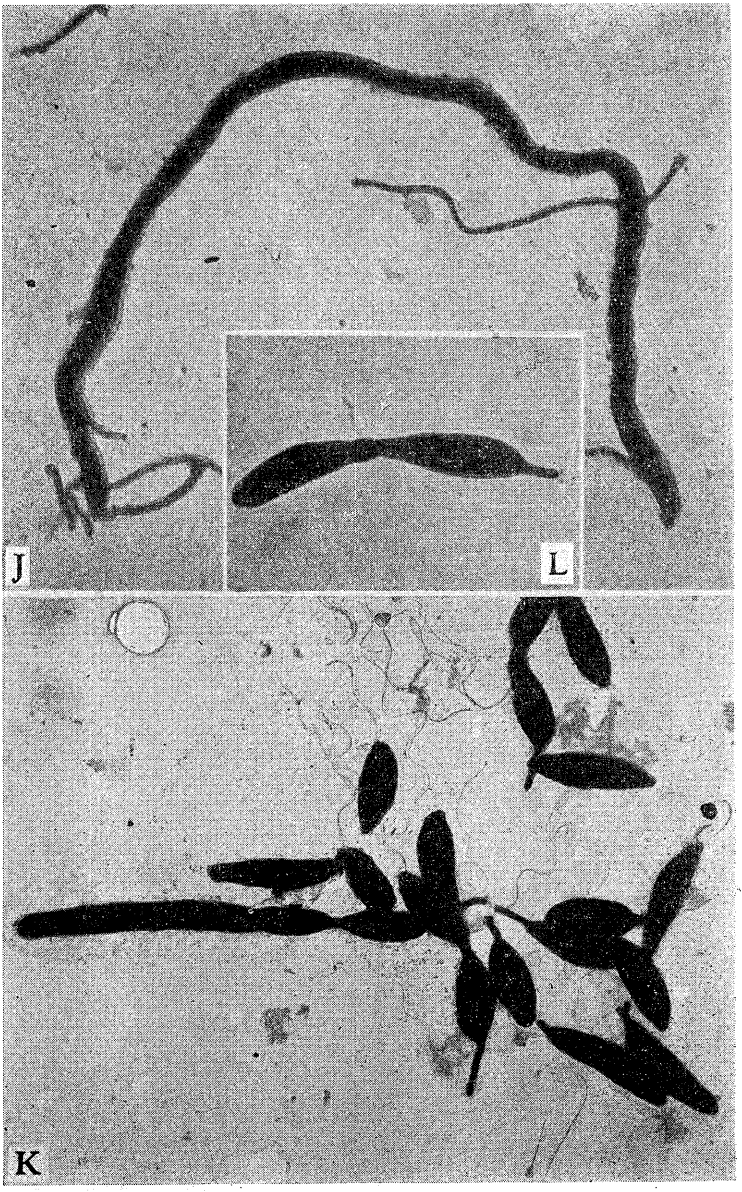


Pl. II. F. Electronmicrograph, chromshadowing, *ca* ×10,000. The pole of stalks touches other pole.

G. Electronmicrograph, chromshadowing, *ca* ×8,500. The cell with two stalks, old and new, at the both end.

H. Electronmicrograph, chromshadowing, *ca* ×8,500. Two cells connected with new stalk.

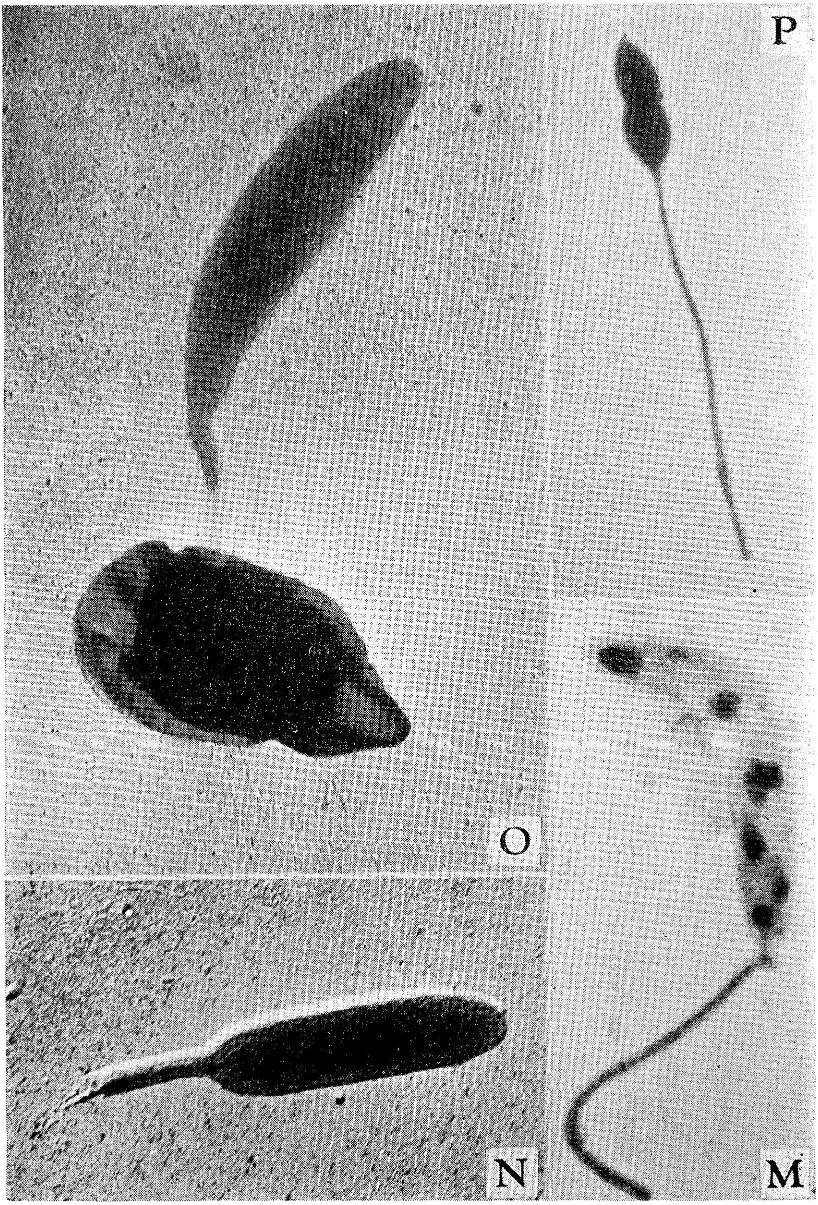
I. Electronmicrograph, chromshadowing, *ca* ×8,500. The multiplication seems to occur soon.



Pl. III. J. Electronmicrograph, chromshadowing, *ca* ×10,000. Filamentous cell in old culture.

K. Electronmicrograph, chromshadowing, *ca* ×10,000. The cells which is multiplying or has multiplied, in young culture which the filamentous cell was inoculated on pepton agar.

L. Electronmicrograph, chromshadowing, *ca* ×10,000. A type of cell which is multiplying.



Pl. IV. M. Electronmicrograph, chromshadowing, *ca* ×13,000. In the stalk, the central filament is seen.

N. Electronmicrograph, chromshadowing, *ca* ×13,000. The outgrowth of stalk was broken and the central filament exposed.

O. Electronmicrograph, chromshadowing, *ca* ×22,000. *Caulobacter* was parasitic on *B. magatherium.*

P. Electronmicrograph, unshadowing, *ca* ×10,000. *Caulobacter* in well water.